PIP2 activation of a voltage-dependent potassium channel (Kv7.1). Kv7.1 channels have a canonical Kv structure with a central pore-gate domain (PGD) surrounded by four peripheral voltage-sensing domains (VSDs). Kv channel activation is thought to involve two general steps. First, membrane depolarization is sensed by gating charges in the VSDs resulting in conformational changes within the VSDs from the resting state to the activated state. After all four VSDs have been activated, the second general step in Kv activation involves a concerted motion during which the PGD opens to allow ion permeation. In this study we ask if PIP2 regulates activation of Kv7.1 by potentiating the early VSD conformational changes, the concerted opening of the PGD, or both. Using the voltage-clamp fluorometry (VCF) technique to assay local conformation changes in the VSDs and the PGD simultaneously, we are able to show that although depletion of PIP2 eliminates the ionic current, the early conformational changes in the VSD do not require PIP2. This result indicates that the later conformational changes that couple VSD activation to the opening of the PGD are eliminated by PIP2 depletion. We continue this work by dissecting the molecular details of why PIP2 is required for opening of the PGD in response to VSD activation. These results may provide insights on the common principle of how Kv channels are modulated by PIP2.

1684-Pos Board B454
Activation of Voltage Sensing Domains during KCNQ1 Channel Opening: Concerted or Independent?
Rene Barro-Soria[1], Jeremiah D. Osteen[2], Kevin J. Sampson[2], Marta E. Perez[1], Robert S. Kass[3], Peter Larsson[1].
[1]University of Miami, Miami, FL, USA, [2]Coumbia University, New York, NY, USA, [3]Columbia University, New York, NY, USA.
In the heart, Iks current is a major contributor in limiting the duration of the action potential. The Iks channel consists of four α-subunits (KCNQ1) which assemble with β subunits (KCNE1). Mutations in either KCNQ1 or KCNE1 cause multiple cardiac arrhythmia syndromes such as LQT syndrome, short QT syndrome and familial atrial fibrillation. Iks channels, characterized mainly by its slow activation and deactivation kinetics and opening at depolarized voltages, differ from those of functional tetrameric voltage-gated KCNQ1 channels expressed alone: fast activating and deactivating kinetic and opening at less depolarized voltages. Understanding the channel structure-function relationship represents a valuable tool in predicting not only cardiac arrhythmia risks, but most importantly possible therapeutic solutions. We aim to unveil the molecular mechanism underlying channel opening in wild type (wt) KCNQ1 channel and its interaction with KCNE1, as well as the molecular mechanism underlying arrhythmia-inducing mutations. To date, two models have been proposed for KCNQ1 channel activation: 1) a cooperative S4 movement, in which the channel opening occurs after a coordinated S4 voltage-sensing domain movement and 2) S4 moves independently to each other concomitantly rendering a step-wise current activation. To test the validity of these models, we characterize the electrophysiological properties of natural occurring LQT syndrome mutations such as R231 in KCNQ1 channel using two electrode voltage clamp (TEVC) and voltage clamp fluorescence (VCF) techniques. R231A mutation has been shown to cause KCNQ1 channel constitutively activated, probably by keeping S4 voltage-sensing domain locked in the activated state. We performed TEVC and VCF measurements of tetrameric constructs of R231 combined with wt KCNQ1 channel to determine whether the S4 voltage-sensing domains in KCNQ1 move independently of each other. Our data will provide insight into the mechanism by which KCNQ1 channel operates.

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N and C Terminal Interactions Underlie Channel Gating of M Channels
Sivan Siloni, Adi Etzioni, Dodo Chikvashvili, Ilana Lotan.
Tel Aviv university, Tel Aviv, Israel.
Neuronal M-type K channels composed of KCNQ2 and KCNQ3 subunits regulate firing properties of neurons. Presynapticlly, KCNQ2 subunits were also found to regulate neurotransmitter release by directly influencing presynaptic function. Previously, we have showed the existence of constitutive interactions between the cytosolic N and C termini of homomeric KCNQ2 or KCNQ3 channels in living cells, and demonstrated that a modulation of the N-C interactions of KCNQ2, but not of KCNQ3, by two regulatory proteins, syntaxin 1A and calmodulin, accompany a reduction in single-channel open probability, suggesting that closer N-C termini proximity underlies gating downregulation. Furthermore, in KCNQ3, identified N terminal and C terminal structural determinants, employ the preclusion of the regulatory proteins effects on the N-C rearrangement and gating regulation. This strongly suggested that N and C structural determinants confer the isoform-specific gating downregulation. Notably, the N-C interactions of both subunits were found to consist of a basal interaction, similar in both channels, and an additional interaction in KCNQ3 formed by an NT distal-end module. Here, we show that the basal N-C interaction is common to both KCNQ2 and KCNQ3 subunits and is essential for proper channel gating of both types of channel. We demonstrate, using optical, biochemical, electrophysiological and molecular biology analyses, that mutations and truncations at specific locations at the N or C termini of the channels abolish, partially or totally, the channels N-C interaction, as well as single-channel gating. Further, we look into the involvement of calmodulin in the basal N-C interactions.

1686-Pos Board B456
Allosteric Properties of KCNQ1 (Kv7.1) Channel Gating Detected by Voltage Clamp Fluorometry
Jeremiah D. Osteen[1], Seth H. Robey[1], Rene Barro[2], Santiago Rebolledo[2], Kevin J. Sampson[1], Hans P. Larsson[2], Robert S. Kass[1].
[1]Columbia University, New York, NY, USA, [2]University of Miami, Miami, FL, USA.
KCNQ1 (Kv7.1) is a unique member of the superfamily of voltage gated K+ channels in that it displays a remarkable range of gating behaviors tuned by co-assembly with different β subunits of the KCNE family of proteins. Homomeric KCNQ1 channels activate quickly over a negative range of voltages; KCNQ1/KCNE1 channels activate very slowly over a depolarized range of voltages; and KCNQ1/KCNE3 channels are constitutively open. To better understand the basis for the biophysical diversity of co-assembled channels, we here investigate the basis of KCNQ1 gating in the absence of β subunits using voltage clamp fluorometry (VCF). Based on our previous work, the kinetics and voltage dependence of voltage sensor movements are very similar to those of the channel gate, suggesting a one-to-one relationship. Here, we have tested alterative hypotheses to explain KCNQ1 gating: 1) KCNQ1 voltage sensors undergo a single concerted movement that leads to channel opening, or 2) independent voltage sensor movements lead to channel opening before all voltage sensors have moved. We find that KCNQ1 voltage sensors move independently, but that the channel can conduct before all voltage sensors move. In some mutant KCNQ1 channels, transition to the open state occurs even in the absence of voltage sensor movement. In these mutants, voltage sensors display more depolarized voltage dependence than the channel gate, implying that voltage sensors move after the channel has opened. To interpret these results, we propose an allosteric gating scheme wherein KCNQ1 is able to transition to the open state after 0-4 voltage sensor movements, with each successive voltage sensor movement strengthening the opening transition. This model allows for widely varying gating behavior depending on the relative strength of the opening transition, which physiologically is controlled by co-assembly with different KCNE family members.

Channel Regulation & Modulation II

1687-Pos Board B457
Characterization of the Role of PKC-Theta in the Modulation of ClC-1 Chloride Channel Function and Calcium Homeostasis in Fast- and Slow-Twitch Skeletal Muscle by using PKC-Theta Null Mice
Sabata Pierno[1], Antonella Liantonio[1], Giulia M. Camerino[1], Michela De Bellis[1], Maria Cannone[1], Maria Maddalena Dinardo[1], Antonia Scaramuzzi[1], Luca Madaro[2], Marina Bouchè[2], Jean-François Desaphy[1], Diana Conte Camerino[1].
[1]University of Bari - Dept PharmacoBiology, Bari, Italy, [2]Sapienza University - Dept Anatomical, Histological, Forensic, Orthopaedic Sciences, Roma, Italy.
In skeletal muscle the resting chloride conductance (gCl), sustained by the ClC-1 chloride channel, controls membrane electrical stability as its absence causes myotonia. The gCl is typically higher in fast-twitch than in slow-twitch muscle and is negatively modulated by Protein Kinase C (PKC). Different PKC isoforms are expressed in skeletal muscle, including the PKC-theta. In PKC-theta-null mice (Sun et al., *Nature,* 2000) we found a significant increase in gCl of slow-twitch soleus muscle with respect to wild-type, being 1876 ± 53 $\mu S/cm^2$ (n=41) and 1356 ± 37 $\mu S/cm^2$ (n=19), respectively. A minor 13% increase of gCl was found in the fast extensor digitorum longus (EDL) muscle. Muscle excitability was reduced accordingly. Chelerythrine, a non-specific PKC inhibitor, further increases gCl by 25% in transgenic soleus muscle showing that other PKC isoforms are involved in the control of gCl. Preliminary experiments suggest indeed an up-regulation of the PKC-alpha isoform in these mice. Minor effect of chelerythrine was found in EDL of PKC-theta null mice. Fluvastatin, known to activate PKC (Pierno et al., *Br J Pharmacol,* 2009) reduced gCl in EDL muscle more than in soleus muscle of transgenic mice confirming that other PKC isoforms contribute to ClC-1 modulation. No modification of ClC-1 expression was found in soleus and EDL muscle of PKC-theta-null mice compared to wild-type. In these mice we also found